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QUINE INTELLECTUAL PROPERTY LAW GROUP, P.C.

By: Deborah Barragan
Deborah Barragan

Appl. No. : 10/536,885 Confirmation No. 6613

Applicant : Ebrahim Firoozabady, et al.

Filed : May 31, 2005

TC/A.U. : 1638

Examiner : Russell Kallis

Docket No. : 63-000600US

Customer No. : 22798

Client Ref No.:

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**DECLARATION OF DR. EBRAHIM FIROOZABADY PURSUANT TO 37 C.F.R.
§ 1.132**

I, Dr. Ebrahim Firoozabady declare:

I am an expert in the field of plant transformation. My C.V. is attached.

I have read the Final Office Action of December 4, 2008. It is my opinion that the Examiner has misinterpreted the main reference by Mezzetti et al. (BMC Biotechnology (2002) pp.1-10). In fact, Mezzetti teaches essentially the *opposite* of what is being claimed.

Specifically, the method described by Mezzetti et al consists of inducing meristematic bulks by long term culture of shoot meristems with repeated mechanical treatment (continuous removal of meristems) and slicing of the tissues very thinly and repeatedly for three months, then maintaining and subculturing these monthly until used for transformation (see Figures 1, 2A, 3 and 4). Everyone familiar with the art of tissue

culture will appreciate that, after going through such long culture and other procedures, the tissues go through undifferentiation and production of non-organized tissues (callus). Indeed, the thin slices in Fig 3A of Mezzetti clearly show this. The Figure shows that the tissues consists of callus and organized meristematic regions.

Furthermore, Mezzetti et al. expressly distinguish their method from direct organogenesis from lateral auxiliary shoots or leaf, arguing that the slicing and culturing of the meristematic bulk (which, as noted above, results in callus) is "critical" to their methods. Page 5, column 2. Not only is Mezzetti teaching a method that is essentially the opposite of what the subject application claims, *one of skill would have been lead to believe from Mezzetti that that the claimed methods of the invention would not work.*

Our method, in contrast, uses direct regeneration of transformed shoots from culture of leaf bases. The leaf bases produce shoots within a few weeks without any callus formation. The primary reason that this works is that leaf bases have lateral meristems and meristematic tissues due to in vitro culture.

The attached pictures in Appendix A help illustrate our unique method.

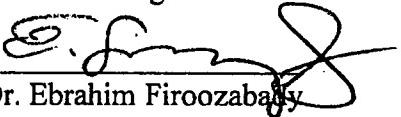
As shown in the figures, leaf bases are prepared, inoculated with Agrobacterium and after cocultivation are exposed to selection. During the selection, original leaf bases turn brown and die. However, meristems produce transformed shoots directly without any callus. The shoot meristems developed at the base of the leaves have a high capacity for transformation.

The pictures of Appendix A further illustrate our method: **Fig 1.** Leaf bases after separation from the shoot have meristems attached to them or have meristem regions developed at their bases. **Fig 2 to 4.** The meristems develop into shoots directly. **Fig. 5.** A GUS assay on one of the meristems, which shows transformation.

I further declare that:

All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Declarant's signature:


Dr. Ebrahim Firoozabady

4/6/09

Date

Appendix A

Fig 1. Leaf bases after separation from the shoot have meristems attached to them or have meristem regions developed at their bases.

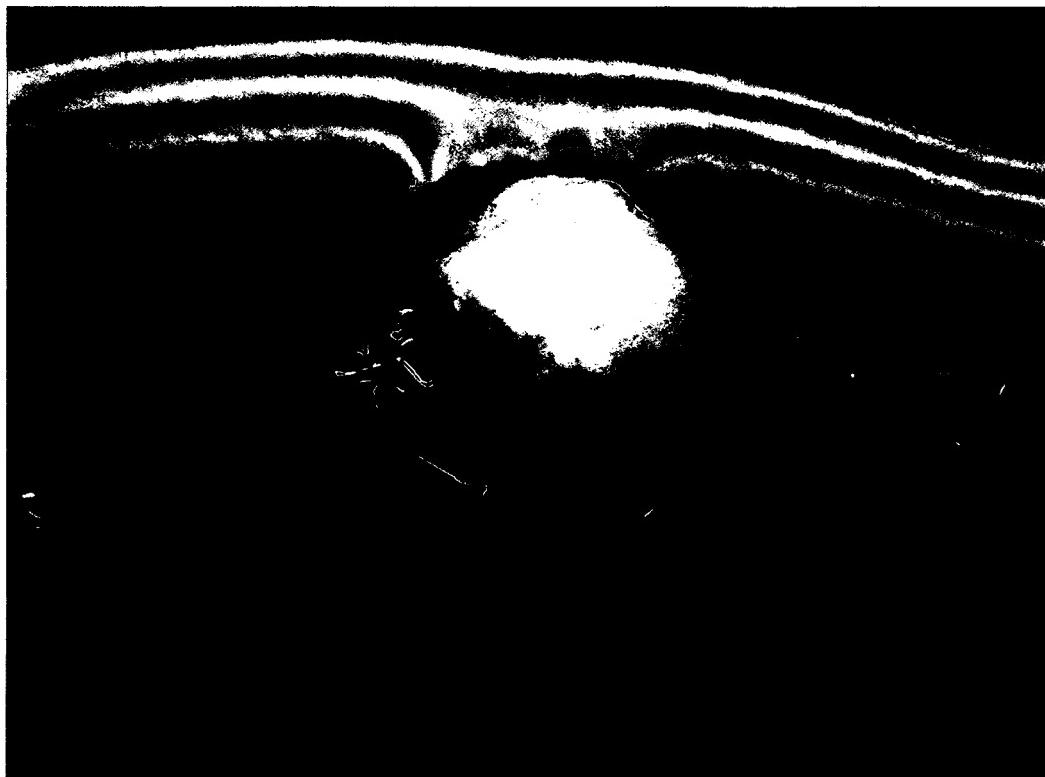
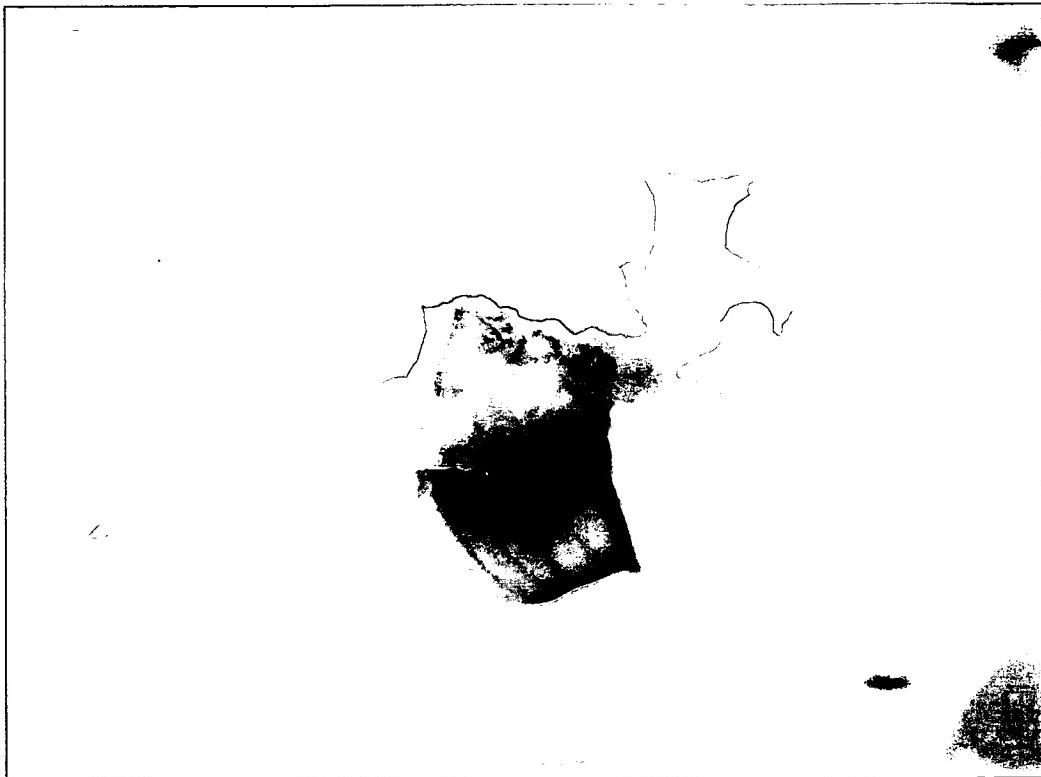


Fig 2 to 4: The meristems develop into shoots directly



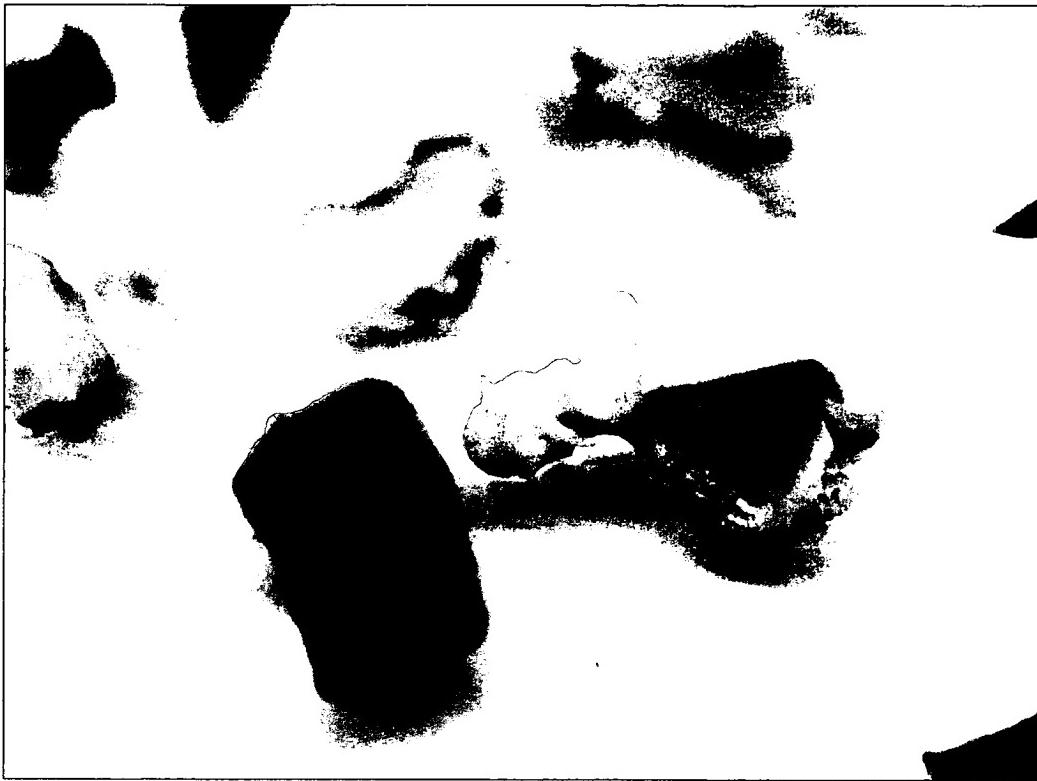
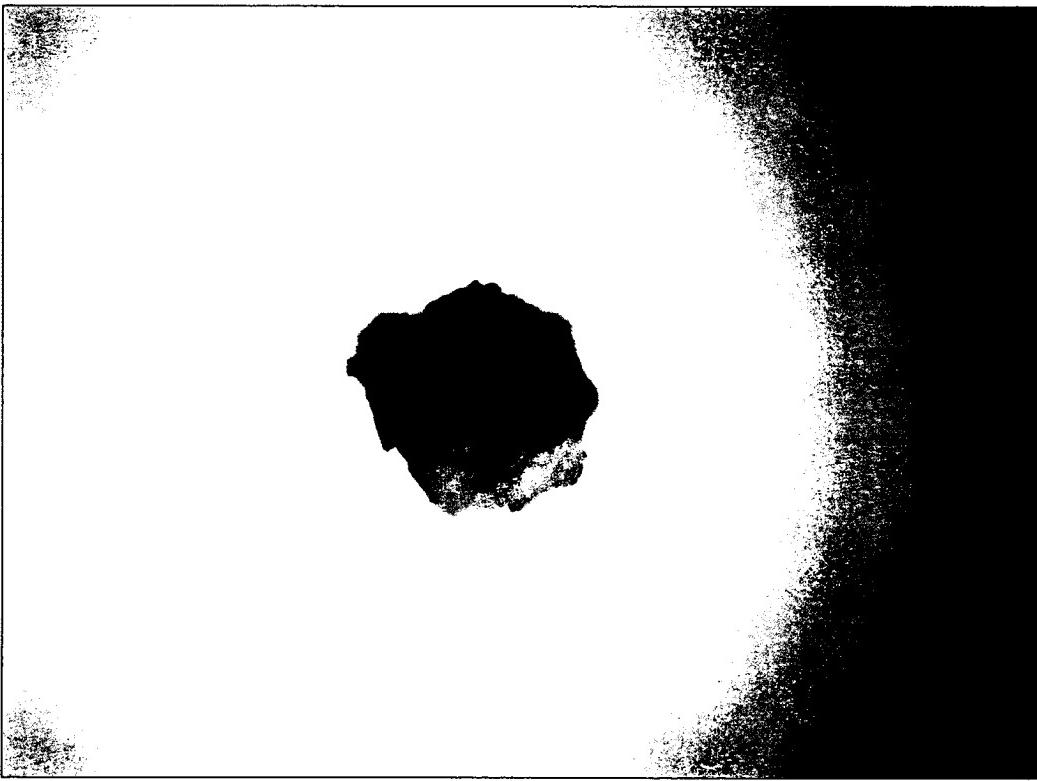


Fig. 5 The GUS assay on one of the meristems, which shows transformation.



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EDUCATION:

UNIVERSITY OF CALIFORNIA, Davis, California
Ph.D., Genetics, 1982

UNIVERSITY OF CALIFORNIA, Davis, California
M.S., Vegetable Crops, 1978

UNIVERSITY OF TEHRAN, Iran
B.S. in Plant Protection (Plant Pathology and Entomology),
1975

WORK EXPERIENCE:

- 6/2005-present Sr. Director, Research and Development Laboratories, Del Monte Fresh Produce Company, Richmond, California and Costa Rica.
- 6/2002-6/2005 Director, Research and Development Laboratories, Del Monte Fresh Produce Company, Richmond, California and Costa Rica.
- 1989 – 5/2002 Principal Research Scientist, DNA PLANT TECHNOLOGY CORPORATION, Oakland, California
- 1989-1992 Group Research Scientist, DNA PLANT TECHNOLOGY CORPORATION, Development of transformation/regeneration methods for banana, pineapple, papaya, palms, rose, carnation, and chrysanthemum. Producing genetically engineered plants listed above containing desirable transgenes. Studies of gene expression in transgenic plants.
- Jan - June 1989 College Professor, NEW MEXICO State University, Las Cruces, NM Producing genetically engineered cotton and alfalfa plants. Giving lectures to a plant molecular biology course.

- 1987-1988 Senior Research Scientist AGRIGENETIC ADVANCED SCIENCE CO., Madison, Wisconsin, Senior Research Scientist Continuation of the research work as outlined below for 1984 -1987. Also, as the group leader of eight researchers involved in transformation studies, we have been successful in developing protocols for transformation and regeneration of potatoes, cotton, sunflower, alfalfa, rape seed, tobacco, tomatoes and potatoes. In this content, cotton, sunflower, tobacco and tomatoes have been directly under my supervision. I have been involved in application for field trials, regulatory issues and field trial of transgenic tomatoes.
- 1982-1984 Research Scientist, AGRIGENETIC ADVANCED SCIENCE CO., Madison, Wisconsin, Developed transformation procedures for cotton, tobacco, potato, tomato, B. napus, alfalfa and sunflower A) Developed transformation procedures (using *Agrobacterium tumefaciens*) for cotton and tobacco; B) developed an efficient plant regeneration system for cotton and sunflower applicable to transformation; C) studied gene expression and inheritance in transgenic plants; D) developed a rapid plant regeneration scheme for protoplasts and leaf disks of tobacco; and E) developed a protocol for protoplast isolation and culture in cotton. *Agrobacterium*-mediated transformation techniques: cotyledon71leaf-disk and stem-hypocotyl inoculation, protoplast-bacterial cocultivation.
- 1984-1987 Post-Doctoral Research Associate in David Galbraith's Lab UNIVERSITY OF NEBRASKA - LINCOLN, Post-Doctoral Involved in both molecular and cellular biology. Anther culture and haploid regeneration in *Nicotiana* spp. Protoplast isolation, culture, and fusion in *Nicotiana*. Transfer of *Agrobacterium* genes to plant protoplasts by (1) cocultivation of bacteria and protoplasts, (2) fusion of protoplasts with plasmids, and (3) isolation of DNA, encapsulation of DNA within liposomes, and subsequent fusion of the liposomes with protoplast. Studies on the effects of cell wall and cell cycle on the process of transformation and crown gall tumorigenesis.
- 1981 Cornnuts Inc. Salinas, California. Breeding squashes, pumpkins and sweet corn to improve for economic traits.
- 1977-82 University of California-Davis. Graduate Research Assistant, Breeding table grape cultivars for performance. Heritability and correlation studies of quantitative traits in table grapes. Selection resistance to root-knot nematode and grape phylloxera. Breeding isozyme variation studies in tomatoes.
for
and
- 1975-76 University of Tehran-Iran, Plant Pathologist, Plant Protection Department. Screening for virus-free potatoes via meristem culture.

1974-75 University of Tehran-Iran, Undergraduate Research, Plant Protection Department. Screening for native potato cultivars resistant to Fusarium oxysporum.

PATENTS/INVENTIONS

Wintz, H-C and Firoozabady, E. 2005, patent PUBLICATION NUMBER- 2006113709/WO-A2 Plant promoters, terminators, genes, vectors and related transformed plants.

Firoozabady, E. WO 2004/053082, PCT/US2003/038912. Organogenic transformation and regeneration

Young, T. and Firoozabady, E. WO 2004/052085, PCT/US2003/038664. Transgenic pineapple plants with modified carotenoid levels and methods of their production

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Firoozabady, E. and Gutterson, N. Issued 1999. Genetically transformed pineapple plants and methods for their production. USA Patent No. 5,952,543

Firoozabady, E. and Gutterson, N. Issued 1998. Genetically transformed pineapple plants and methods for their production. Int'l Patent No. WO 98/36637

Firoozabady, E. Issued 1998. Genetically transformed rose plants and methods for their production. US Patent no. 5,792,927

Firoozabady, E. and K. Robinson. Issued 1996. Rose plants and methods for their production and genetic transformation. US Patent no. 5,480,789

Firoozabady, E. et al. Issued 1996. Carnation plants and methods for their transformation and propagation. USA Patent No. 5,589,613

Firoozabady, E. et al. Issued 1992. Carnation plants and methods for their transformation and propagation. Int'l Patent No. WO 92/17056

Firoozabady, E. and K. Robinson. Issued 1992. Rose plants and methods for their production and genetic transformation. Int'l Patent no. WO 92/00371

Firoozabady, E. and C. Lemieux. Issued 1992. Methods for producing transformed chrysanthemum plants. Int'l Patent no. WO 92/xxxxx

Power, C. and E. Firoozabady. Issued 1991. Sunflower regeneration from cotyledons. US Patent No. 5,030,572

Firoozabady, E. US Patent Serial No. 076,339. Cotton regeneration and transformation.

ACTIVITIES AND MEMBERSHIPS IN PROFESSIONAL SOCIETIES

Vice-president Plant Division of the Society for In Vitro Biology (SIVB) formerly Tissue Culture Association, 1994-96.

Chairman of the symposium "cell cycle/cell death" in the annual congresses of the SIVB, 2000

Chairman of the symposia in the annual congresses of the SIVB, 1993-1998.

Member of the planning committee of Plant Division of SIVB, 1991-present.

Member of the SIVB, 1989-present.

Member of the American Society for Horticultural Science, 1989-1997.

Member of the International Society for Plant Molecular Biology, 1988-1994

Recently was appointed to serve as an Editor, for the international journal of Plant Cell Reports.

AWARDS AND RECOGNITIONS

- a. I was nominated (by the committee) for the 2002 Noble Prize for Outstanding Achievement and Contributions to Humanity . This is not the famous Nobel Prize but it is called Noble Prize and hence this prize was only \$100,000 instead of \$1,000,000 for Nobel Prize. This new prize was established in 2001 and I was nominated for my work at Agrigenetics, during years of 1984-89, for developing insect resistant tomato and cotton. I was one of the first few scientists in the world to develop insect resistant plants via genetic engineering. This principle has now been applied to many crops around the world and has increased productivity in many developing countries.
- b. Recipient of \$6 million grant to DNAP Company by Thailand Pineapple Company to produce genetically engineered pineapples with three novel traits (1992-1998).
- c. Recipient of \$1.8 million grant to DNAP Company by the United States Agency for International Development to develop large-scale micropropagation methods for tropical crops grown in developing countries. This project was very successful as I developed the methods and trained the scientists from Costa Rica, Thailand and Indonesia (1991-1998).
- d. Professor Award, New Mexico State University, Las Cruces, to study and produce genetically engineered cotton and alfalfa plants, 1989.
- e. Recipient of \$400,000 research contract grant to Univ. of Nebraska by Agrigenetics Company to produce genetically engineered cotton plants, 1983-86 (but left the University in 1984).

- f. Recipient of graduate program international research award by UC-Davis while working toward Ph.D. degree, 1978-82.
- g. Recipient of Industrial University of Isfahan scholarship award for continuation of education toward MS and Ph.D., 1976-82.
- h. Recipient of University of Tehran scholarship award for continuation of education toward BS, 1971-75.
- i. Recipient of first place student awards in University of Tehran undergraduate program (1972-75) and high school (1966-71).
- j. I was invited by The United Nations to participate in an international conference on Plant Biotechnology in Tehran, Iran (1999).
- k. I was invited by The United Nations to have a workshop on Plant Transformation and Biotechnology in Karaj. This was a workshop for two weeks; training ~100 Iranian scientists (BS, MS and Ph.D. levels) representing different institutions in Iran, 1997.
- l. I have been recognized as a leader scientist in new eras of science and my biographical profile has been published in several key reference books including *2000 Outstanding Scientists of the 20th Century*, *Who's Who in Science and Engineering*, *Who's Who in the World*, *Who's Who in America*, and *Who's Who in the West*.
- m. I have been invited to many international conferences to speak.
- n. Some of my studies have made significant contributions to the field and have been recognized internationally and been given citations for:
 - a. Blue genes rose will bloom in two years. The Daily Telegraph, London May 30 1994.
 - b. Gene swap may lead to the £50 blue rose. The Daily Telegraph, London May 30 1994.
 - c. Advances in Plant Regeneration from Tissue Cultures Improved medium speed regeneration of plants from Protoplast. (1987) Agricell Report 8:9-11.
 - d. Genetic Engineers Promise Pest Resistant Varieties. (1987) Progressive Farmers 102: cotton 12 - cotton 13.
 - e. Plant Transformation Advances - Are cell walls required for plant transformation? (1984) Agricell Report 3:10.

FELLOWSHIPS AND AWARDS

Recipient of scholarship award for continuation of education toward MS and Ph.D., 1976 - 1982.

Recipient of scholarship award for continuation of education toward BS, 1971 - 1975.

Recipient of first place student awards in undergraduate program (1972 - 1975) and high school (1966 - 1971).

TEACHING EXPERIENCE

College Professor New Mexico State University, Las Cruces, Gave invited lectures in a plant molecular biology course, 1989.

Instructor, Southeast Community College, Lincoln, NE. January, 1984 to May, 1984.

Invited speaker: University of Nebraska-Lincoln, Gave invited lectures in different plant biology courses, 1982-1984.

Tutor. Genetics, Learning Skills Center, University of California, Davis. January, 1981 to March, 1982.

Teaching Assistant. Principles of Genetics, Genetics Department, University of California, Davis. January, 1979 to September, 1979.

Teacher. Karaj High School. June 1973 to August 1973.

Others:

- a. Over the last 10 years at DNAP have trained 16 students from UC Berkeley as Research Lab Assistants.
- b. Have given seminars at the meetings, universities, junior colleges and high schools.

INSTITUTIONAL AND ACADEMIC SERVICES

Member, Institutional biosafety committee, DNAP, 1999-present.

Chairman, Seminar speakers committee, DNAP, 1996-1998.

Chairman, Library Committee, Agrigenetics, Madison, WI, 1984 to present.

Member, Graduate Student Association for Genetics, Topics Selection Committee, 1978 to 1981.

Member, Graduate Student Association for Vegetable Crops, Laboratory and Facilities Use Committee, 1977 to 1978.

PUBLICATIONS:

Firoozabady, E., M. Heckert, and N. Gutterson. Transformation and regeneration of pineapple, *Plant Cell, Tissue and Organ Culture* (2006) 84: 1-16

Firoozabady, E., Y. Moy and D. Engler Transformation and regeneration of banana cv. Williams. SIVB 2001

FIROOZABADY E, Y. Moy, P. Oeller and N. Gutterson. Improvement of transformation and regeneration in papaya, in preparation.

Trusov, Y., Firoozabady, E., Paul Oeller, N. Gutterson and J. Botella. Control of flowering in pineapple via genetic engineering.

Firoozabady E. and N. Gutterson. Cost-effective in vitro propagation methods for pineapple, *Plant Cell Reports* 2003

Firoozabady, E., and Y. Moy. Regeneration of pineapple plants via organogenesis and somatic embryogenesis, *In Vitro Cell. Dev. Biol.*—*Plant* 40:67–74, 2004

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Firoozabady E., M. Heckert, and N. Gutterson, 1997. Transformation and regeneration of transgenic pineapple plants. Abstract of the 1197 Congress of the Society for in vitro Biology, Washington DC., June 1997 (invited speaker)`.

Firoozabady E., M. Heckert, Paul Oeller, and N. Gutterson, 1997. Transformation and regeneration of transgenic pineapple plants. Abstract of the 5th Congress of the Society for Plant Molecular Biology, Singapore, 1997.

Firoozabady E., J. Nicholas and N. Gutterson. 1996. *In vitro* plant regeneration and advanced propagation methods for pineapple. Proceedings of the Second International Symposium on Pineapple. Martinique

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Firoozabady E., Y. Moy, N. Courtney-Gutterson, and K. Robinson. 1994. Regeneration of transgenic rose (*Rosa hybrida*) plants from embryogenic tissue. *Bio/Technology* 12:609-613.

Courtney-Gutterson, C. Napoli, C. Lemieux, A. Morgan, E. Firoozabady, and K. Robinson. 1994. Modification of Flower color in Florist's chrysanthemum: production of a white-flowering variety through molecular genetics. *Bio/Technology* 12:268-271.

Robinson, K. and E. Firoozabady, 1993. Transformation of Floriculture Crops. In: *Genetic Engineering of Ornamental Crops* Scientia Hort., 55:83-99.

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Robinson, A. Otten, and M. Akerboom. 1993. Production of genetically engineered color-modified chrysanthemum plants carrying a homologous chalcon synthase gene and their field performance. *Acta Horticulturae* 336:57-62.

Murray, E.E., D.L. DeBoer and E. Firoozabady, 1993. Transgenic cotton, in *Transgenic Plants*, Kung, S. and R.Wu (eds.). Academic Press, Vol. 2:153-168.

Firoozabady E., and D.L. DeBoer. 1993. Plant regeneration via somatic embryogenesis in many cultivars of cotton (*Gossypium hirsutum* L.), **In Vitro**: 29P:166-173.

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Adang, M.J., D. DeBore, J. Endres, E. Firoozabady, et al. 1989. In "Biotechnology, biological pesticides and novel plant-pest resistance strategies for insect pest management" (eds Roberts, D. W., and R.R. Granados). Expression of a *Bacillus thuringiensis* insecticidal crystal protein gene in tobacco plants. pp 31-37. Boyce Thompson Institute for Plant Research, Ithaca, New York.

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Firoozabady, E. and D.L. DeBoer, 1986. Isolation, culture, and cell division in cotyledon protoplasts of cotton (*Gossypium hirsutum* and *G. barbadense*). **Plant Cell Reports** 5:127-131.

Firoozabady, E., D.L. DeBoer, and M. J. Maroney. 1986. Transformation and regeneration of cotton, *Gossypium hirsutum* L. in Tailoring genes for crop improvement, UCDavis pg 24.

Firoozabady, E., 1986. The effects of cell cycle parameters on cell wall regeneration and cell division of cotton protoplasts (*Gossypium hirsutum* L. *Journal of Experimental Botany* 37: 1211-1217.

Firoozabady, E., 1986. Rapid plant regeneration from *Nicotiana* mesophyll protoplasts. *Plant Science* 46:127-131.

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Firoozabady, 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. *Science* 220: 1049-1051.

Firoozabady, E and D.W. Galbraith, 1984. Presence of a plant cell wall is not required for transformation of *Nicotiana* by *Agrobacterium tumefaciens*. *Plant Cell, Tissue and Organ Culture* 3:175-188.

Firoozabady, E. and D.W. Galbraith, 1983. The effects of cell wall and cell cycle on crown gall tumorigenesis. *HortScience* 18(4):618.

Sharma, D.P., E. Firoozabady, N. Ayres, and D.W. Galbraith, 1983. Improvement of anther culture in *Nicotiana*: media, cultural conditions and flow cytometry determination of ploidy levels. *Z. Pflanzenphysiol Bd.* 111:441-451.

Firoozabady, E. and H.P. Olmo, 1987. Heritabilities and correlation studies of certain quantitative traits in table grapes, *Vitis* spp. *Vitis* 26:132-146.

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Firoozabady, E., 1982. Heritability and correlation of certain quantitative traits in grapevine, *Vitis* spp. Ph.D. thesis, U.C. Davis.

Firoozabady, E. and H.P. Olmo, 1982. The heritability of resistance to root-knot nematode (*Meloidogyne incognita acrita* Chit.) in *vinifera rotundifolia* hybrid derivatives. *Vitis* 21:136-144.

Firoozabady, E., D.L. DeBoer, E.E. Murray, D.J. Merlo, and E.L. Halk, 1987. Transformation of cotton by *Agrobacterium tumefaciens* and regeneration of transgenic plants. *In Vitro* 23:67A.

Firoozabady, E.**, D.L. DeBoer, D.J. Merlo, E.L. Halk, L. Amerson, and E.E. Murray, 1987. Transformation of cotton (*Gossypium hirsutum* L.) by *Agrobacterium tumefaciens* and regeneration of transgenic plants. Int'l. Cong. Plant Tissue Culture - Tropical Species, Bogota, Columbia, pp.30-31.

Firoozabady, E., D. Merlo, G. Staffeld, D. DeBoer, N. Reichert, and H. Stratton, 1985. Expression and biochemical studies of neomycin phosphotransferase II gene in transformed tissues of *Nicotiana tabacum* c.v. Xanthi. 1985. First Int'l. Cong. Plant Molecular Biology, Savannah, GA p. 115.

Firoozabady, E. and D.W. Galbraith, 1985. Effects of cell cycle on transformation of Nicotiana protoplasts by Agrobacterium tumefaciens. Biotechnology in Plant Science: Relevance to Agriculture in the Eighties, Cornell University, Ithaca, NY.

Firoozabady, E., DeBoer, D.L., Klein, J., Merlo, D.J., Murray, E., Rocheleau, T., Rashka, K., Staffeld, G., Stock, C., and Adang, M.J., 1986. Expression of a Bacillus thuringiensis crystal protein gene in Nicotiana tabacum. Int'l Conf.: Tailoring genes for crop improvement, U C. Davis.

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Firoozabady, E., DeBoer, D.L., and Maroney, M.J., 1986. Transformation and regeneration of cotton, Gossypium hirsutum L. Int'l Conf.: Tailoring genes for crop improvement, U.C. Davies p. 24.

Firoozabady, E., D.L. DeBoer, M.J. Maroney, 1986. Transformation and regeneration of cotton, Gossypium hirsutum L. 6th International Congress of Plant Tissue and cell Culture, University of Minnesota, Minneapolis, MN.

Hoffman, L., D. Donaldson, and E. Firoozabady, 1986. Introduction of the maize 15 kd zein gene into dicotyledonous plants via Agrobacterium tumefaciens. EMBO workshop "Plant Storage Protein Genes", Breisach, FRG.

Adang, M.J., D. DeBoer, E. Firoozabady, J.D. Kemp, E. Murray, T.A. Rocheleau, K. Rashka, G. Staffeld, C. Stock, D. Sutton, and D.J. Merlo, 1986. Applications of a Bacillus thuringiensis crystal protein for insect control. Journal of Cellular Biochemistry Supplement 10C:ll.

Adang, M.J., E. Firoozabady, D.L. DeBoer, J. Klein, D.J. Merlo, E. Murray, T. Rocheleau, K. Rashka, G. Staffeld, and C. Stock, 1986. Expression of a Bacillus thuringiensis Crystal Protein Gene in Nicotiana tabacum. VI Int'l. Cong. Plant Tissue & Cell Culture, Minneapolis, MN p.404.

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Merlo, D., E. Firoozabady, J. Klein, E. Murray, D. DeBoer, J. Endres, A. Owens Merlo, K. Rashka, T. Rocheleau, G. Staffeld, and M. Adang, 1988. Insect tolerant plants which express a Bacillus insecticidal protein. Proceedings of the symposium of the Society for Industrial Microbiologists.

REFERENCES:

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